Critical Review

Advances in Evaluating the Oral Bioavailability of Inorganics in Soil for Use in Human Health Risk Assessment

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Cleanup goals for sites affected by inorganic contaminants often are established on the basis of risk assessments, and these assessments rely on the estimated oral toxicity of the substances of concern. These toxicity estimates typically are based on historical studies in which a soluble salt of the metal was dissolved in water or mixed in food and then ingested by an animal or human. However, these toxicity studies do not account for the characteristics of a metal in soil or the limitations that these characteristics place on enteric absorption of that metal. Therefore, a more accurate risk assessment must account for the bioavailability of the metal in site-specific soil, relative to the bioavailability of the metal in the form administered in the toxicity study (i.e., the relative bioavailability of the element in soil). Historically, relative bioavailability estimates for metals in soil have been based on in vivo studies in laboratory animals. Given the costs and time constraints associated with such studies, it is clear that a more efficient alternative is desirable. The most promising option involves the development and validation of in vitro extraction tests that are predictive of oral metals bioavailability from soil. Such tests would provide a rapid and inexpensive method for developing more accurate exposure estimates for use in human health risk assessments. This paper reviews the site-specific in vivo studies that have been conducted to estimate the relative bioavailability of arsenic and lead in soil, discusses the soil and mineralogical factors that influence the bioavailability of these elements, and reviews the research to date on the development of bioavailability-predictive extraction tests for metals in soil. Finally, this paper outlines an ongoing collaborative research project to formally validate an in vitro extraction test for use in estimating the oral bioavailability of arsenic and lead in soil.

Introduction

In this paper, we discuss ongoing research to develop and validate simple extraction tests to estimate the oral bioavailability of metals from soil. Much of our current understanding on this topic derives from in vivo estimates of arsenic and lead bioavailability (i.e., studies in animal models), which have been developed for predicting human exposures to these elements in soil. Given the expense and time constraints associated with in vivo testing, development of accurate and inexpensive extraction tests that are predictive of relative bioavailability provides an opportunity to improve the accuracy of risk assessments in a cost-effective manner. Such tests could be used both for site assessment and for developing and evaluating remedial technologies, like soil washing or soil amendments, that may have an impact on metals bioavailability. Because the extraction tests that have been developed to date can be conducted for a small fraction of the cost of in vivo studies, a sufficient number of samples can be evaluated to fully characterize soils at a site. In the long run, it would be ideal to have simple, bioavailability-predictive extraction tests for all metals of human health concern, so that risk from metals in soil could be assessed based on the site-specific fraction of bioavailable metals.

The focus of this paper is on relative bioavailability after oral exposure, because this route is typically assumed to provide the greatest exposure for metals in soil, and because

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this is the exposure route that has been studied most extensively to date. [Although arsenic is technically a metalloid, the term "metal" is used in this paper to refer to the elements arsenic and lead collectively.] The oral toxicity of metals is typically determined on the basis of studies in which a soluble salt of the metal was dissolved in water or mixed with food. If the toxicity values used in risk assessments of metals in soil are based on studies using soluble forms of the metals, the impacts of soil exposures may be overestimated. For metals, reduced absorption from soil may be due to a combination of factors, including the presence of less soluble species of the metal as well as interactions of dissolved metals with soil constituents (e.g., sorption and precipitation reactions). These factors may limit metal dissolution from soil during passage through the gastrointestinal tract. Absorption would then be reduced, because the primary mechanism of absorption for most metals is via passage of dissolved species across the small-intestinal epithelium (*1*). To account for limited dissolution of metals from soil, it may be important to determine the bioavailability of the element in soil, relative to its bioavailability when it is solubilized in water, to derive more accurate exposure estimates.

Because this document addresses issues related to the bioavailability of metals in soil in human health risk assessment, the definition of bioavailability used throughout is the definition commonly used by mammalian toxicologists.

Definitions

Bioavailability. Oral bioavailability is defined as the fraction of an administered dose that reaches the central (blood) compartment from the gastrointestinal tract. Bioavailability defined in this manner is commonly referred to as "absolute bioavailability", and is equal to the oral absorption fraction.

Relative Bioavailability. Relative bioavailability refers to comparative bioavailabilities of different forms of a substance or for different exposure media containing the substance (e.g., bioavailability of a metal from soil relative to its bioavailability from water), expressed in this document as a relative absorption factor (RAF).

Relative Absorption Factor. The RAF describes the ratio of the absorbed fraction of a substance from a particular exposure medium relative to the fraction absorbed from the dosing vehicle used in the toxicity study for that substance (the term relative bioavailability adjustment [RBA] is also used to describe this factor).

Bioaccessibility. The oral bioaccessibility of a substance is the fraction that is soluble in the gastrointestinal environment and is available for absorption. The bioaccessible fraction is not necessarily equal to the RAF (or RBA) but depends on the relation between results from a particular in vitro test system and an appropriate in vivo model.

The following sections (1) discuss the mineralogy and soil factors that influence the extent of lead and arsenic bioavailability, (2) present an overview of the in vivo studies that have been conducted for lead and arsenic in soil, (3) review the in vitro research that has been conducted to date, and (4) discuss some of the ongoing research on validation of in vitro extraction tests for estimating the oral bioavailability of arsenic and lead. This review is limited to arsenic and lead because relative bioavailability studies (e.g., in vivo studies) have been almost exclusively limited to these two elements. However, many of the issues discussed herein are pertinent to other inorganic elements of human health concern (e.g., beryllium, cadmium, chromium, mercury, and nickel), particularly those that exhibit similar geochemical behaviors to arsenic and lead.

Mineralogic and Soil Factors that Control Bioavailability

Lead and arsenic occur in soil as a complex mixture of solid-phase chemical compounds of varying particle size and morphology. These compounds include discrete mineral phases, coprecipitated and sorbed species associated with soil minerals or organic matter, and dissolved species that may be complexed by a variety of organic and inorganic ligands. The occurrence and relative distribution of an element among these various phases, and the physical relation between the phases and the soil, will control an element's dissolution properties and, hence, its bioavailability. The spatial heterogeneity of these complex mixtures in soil will be reflected by variable metal bioavailability from soil at a site.

Changes in the distribution of an element among these various phases over time resulting from physical and chemical weathering, biological processes, the infiltration of water, and anthropogenic disturbances may change the bioavailability of that element. The importance of these changes for human health risk assessment depends on the relative rate and magnitude of the changes in bioavailability. Factors to consider include the relative stability of the compound released to soil, the potential for chemical or physical alteration of this compound, the likely reaction products (based on soil chemistry), and the likelihood of disturbances that may alter soil chemistry. For example, if highly soluble lead acetate is released to soil, lead will rapidly become incorporated in soil minerals, and its bioavailability will decrease over time. In contrast, little change would be expected in the bioavailability of lead dispersed in high-silica smelter slag (due to its relative insolubility), even after this mining waste had remained in the environment for decades. Therefore, the identity of and potential change in elemental speciation (and thus bioavailability) must be considered in predicting human health risk.

Lead. Lead concentrations in naturally occurring surficial soils range from less than 10 to 700 mg/kg (*2*), with an arithmetic mean of 19 mg/kg for soils in the conterminous United States ($n = 1319$ (*3*)). Lead may occur in soils as native pure mineral phases, such as lead sulfide (PbS), lead sulfate ($PbSO_4$), or lead carbonate ($PbCO_3$). Lead sulfide occurs primarily at mining, milling, smelting, and ore-handling sites. At mining and smelting sites, lead minerals may be encapsulated within other soil mineral grains, such as quartz, which limit its bioavailability. In addition, lead minerals are often present within the matrix of smelter slags and other pyrometallurgical waste materials. Lead sulfate and lead carbonate are commonly found as mineral phases in soils; they can occur through inefficient pyrometallurgical processes, but more commonly, they result from precipitation reactions in soils. Formation of lead sulfate is favored in acidic soils, while lead carbonate is favored in alkaline soils (*4*).

All of the lead forms discussed herein exhibit different rates of lead dissolution, depending on their chemistry and particle size distribution, the mechanism by which they dissolve (e.g., surface reaction or transport-controlled dissolution kinetics), and the geochemistry of the soils in which they are present. Figure 1 provides a schematic of the processes that are believed to control the bioavailability of lead in soil. Soil chemistry—including the presence of anionic species that form complexes with lead (e.g., organic acids, soil organic matter [SOM], phosphate, carbonate, sulfides, chloride, and hydroxide), iron and manganese concentrations, soil pH, cation exchange capacity, and redox conditions—determines the extent to which various dissolution, precipitation, complexation, and adsorption reactions occur for lead in a particular soil.

Lead may also be coprecipitated with, or sorbed to, various mineral phases that result from soil weathering. These

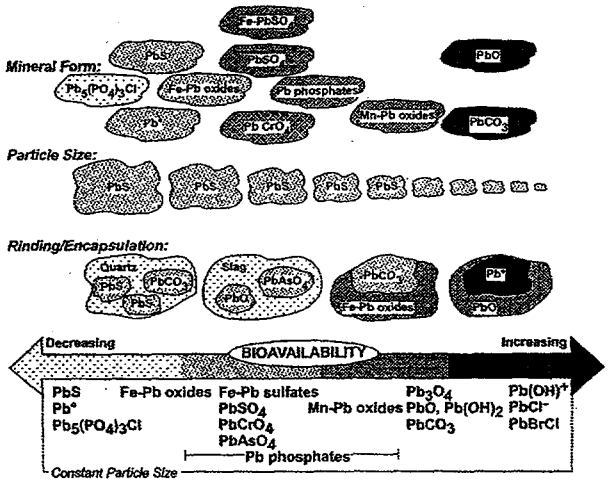

FIGURE 1. Schematic of how different lead species, particle sizes, and morphologies affect lead bioavailability.

minerals—including iron and manganese oxides, iron sulfates, and phosphate minerals—tend to have variable lead compositions and may be found either as discrete mineral particles or as rinds on other soil particles (Figure 1). Mineral phases that form under acidic conditions (e.g., lead sulfate, iron–lead sulfate) will tend to be more stable in the acidic conditions of the stomach and hence less bioaccessible. Mineral phases that form under alkaline conditions (e.g., lead carbonate, lead oxide) will be less stable in the acidic conditions of the stomach and more bioaccessible (Figure 1). The phosphate minerals, in particular, have highly variable compositions (*5*), which result in a wide range of bioaccessibility values. In addition, lead may be present bound to sulfhydryl and carboxyl ligands on SOM, sorbed to clay and other metal oxide (Fe, Al, Mn) mineral surfaces, and as dissolved, complexed ionic species, such as Pb^{2+}, $Pb(OH)^+$, $PbCl^+$, $PbHCO_3^+$, or $Pb(CO_3)_2^{2-}$. Each of these lead species may have a different bioaccessibility, depending on the manner in which it is bound or complexed.

Dissolution rate-controlling processes also are important in determining oral lead bioavailability, because lead must dissolve during the limited transit time in the gastrointestinal tract to become bioaccessible (*6*). Smaller particles have greater ratios of surface area to volume and, hence, are more rapidly solubilized, resulting in greater bioaccessibility and ultimately greater bioavailability (*6–9*). In general, less soluble lead minerals (e.g., lead in calcium phosphates) dissolve by surface-reaction-controlled kinetics, which are limited by surface detachment of ions (*9*). More soluble lead minerals (e.g., lead oxide) dissolve by transport-controlled kinetics, where dissolution ions are detached very rapidly and accumulate to form a saturated solution adjacent to the mineral surface. The dissolution rate thus becomes controlled by the rate of transport of ions away from this saturated layer. The rate-limiting step for dissolution of lead minerals of intermediate solubility (e.g., lead sulfate) is mixed or partial surface-reaction-controlled kinetics (*9*), which contain elements of both the above mechanisms. An understanding of lead mineral dissolution kinetics is important in determining which lead species will contribute to bioavailable lead; the bioavailability of minerals that dissolve through the transport-controlled mechanism is sensitive to the extent of mixing and agitation in the gastrointestinal tract, while the bioavailability of minerals that dissolve through surface-reaction control is sensitive only to transit time.

Soil lead from anthropogenic activities also includes that from paint, gasoline additives, and other commercial products (e.g., lead acid batteries, cable coverings, ammunition, and solder). The most common forms of lead in paint are red lead (Pb_3O_4), which historically was used primarily on painted steel, white lead (also known as basic lead carbonate [$2PbCO_3 \cdot Pb(OH)_2$]), basic lead sulfate ($PbO_2 \cdot PbSO_4$), and lead chromate ($PbCrO_4$ (*10*)). Lead paints were manufactured by drying the lead pigments in sheets, stamping out small (approximately 1–10 μm) particles of the material, and then mixing these particles in a binder material. As a result, as paints weather in soil, they release small particles of lead, which are likely to be highly bioavailable (due to the solubility of lead oxide and carbonate species and the small particle size).

Lead from gasoline used in internal combustion engines (primarily tetraethyl lead) is present as lead halides (e.g., PbBrCl) in fresh exhaust but alters to lead carbonates, oxides, and sulfates due to photochemically induced reactions during atmospheric transport (*11*). As a result, lead from combustion of leaded gasoline is deposited in soils as a mixture of lead halides, carbonates, oxides, and sulfates, which subsequently weather in soil (*11*). When organic lead forms (primarily tetraethyl and tetramethyl lead) are deposited in soils due to gasoline spills, they degrade to inorganic lead with the degradation of the gasoline hydrocarbons (*12*). The resulting lead ions (Pb^{2+}) participate in the soil reactions discussed above.

The lead species present in other commercial products include elemental (native) lead (lead acid batteries, cable coverings, and ammunition), lead sulfate (also present in lead acid batteries), and tin–lead alloys (solder). Elemental lead particles that are deposited in soils quickly form coatings of highly bioavailable lead oxide.

Given the complex and dynamic nature of the soil lead cycle, it is clear that a simple test for estimating lead bioavailability would be useful for quantifying both the extent and rate of change in the bioavailability of lead from soils.

Arsenic. The manner in which arsenic occurs in soils can also be viewed as a complex and evolving mixture. Arsenic concentrations in undisturbed soils range from 0.1 to 97 mg/kg (*2*), with an arithmetic mean concentration of 7.2 mg/kg for surficial soils in the conterminous United States (n = 1257 (*3*)). Arsenic is substantially different from lead, in that it occurs in natural environments in two valence states: arsenic(III) and arsenic(V). Typically, arsenic(III) is present in anoxic conditions, while arsenic(V) is the dominant form of arsenic in oxic soils. Arsenic(III) may occur as uncharged $As(OH)_3$ in acidic soils and as an anion (AsO_3^{3-}) in alkaline soils (*13*). Arsenic(V) is present as an anion ($H_2AsO_4^-$ or $HAsO_4^{2-}$) in the natural pH range of soils (pH 4–8) (*14*). The presence of arsenic as anionic species causes it to be quite mobile in soils when it occurs in a soluble form.

The primary anthropogenic sources of arsenic in the United States include releases from mining and smelting operations, agricultural uses (e.g., pesticides, insecticides, defoliants, feed additives, and livestock dips), tanning operations, wood treatment/preservation operations, and releases as a byproduct of burning coal.

Arsenic, like lead, may occur as sulfide minerals (e.g., arsenopyrite [FeAsS] and enargite [Cu_3AsS_4]) at mining and milling sites. The particle size distribution of the arsenic species discussed herein, in combination with their chemical composition, controls their bioavailability (Figure 2). Arsenic dissolution appears to be particularly sensitive to particle size, especially for certain arsenic mineral phases. In soils, arsenic may be present as the anthropogenic form in which it was deposited (lead and calcium arsenates from pesticides, arsenic pentoxide from herbicides and fungicides, copper-chrome arsenate from wood treating, or arsenic disulfide from tanning operations) or as various soil alteration phases of variable composition, such as arsenic in iron and manganese oxides and in phosphate minerals (arsenate replaces phosphate in the mineral lattice; Figure 2). Arsenic

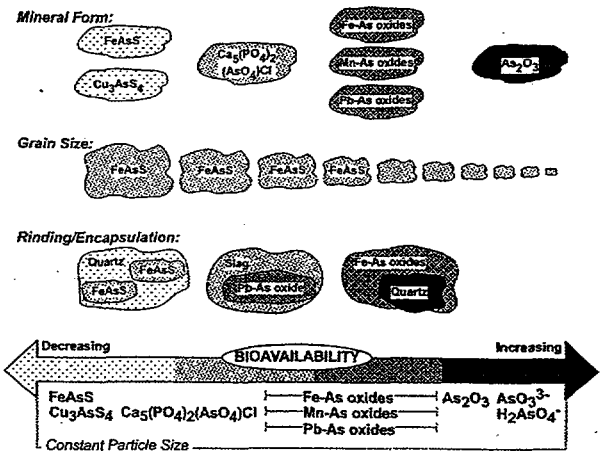

FIGURE 2. Schematic of how different arsenic species, particle sizes, and morphologies affect arsenic bioavailability.

displays a propensity to coprecipitate with iron to form iron–arsenic oxides, the most common soil alteration phase for arsenic (*15*). Arsenic commonly co-occurs with lead in many soil alteration phases, and the same range and complexity of soil lead reactions also take place for arsenic. The in vivo and in vitro studies conducted to date (discussed below) suggest that for a constant particle size, soil arsenic phases such as arsenic sulfides (arsenopyrite [FeAsS] and enargite [Cu_3AsS_4]) and arsenic dispersed in slag have lower relative bioavailability than iron–arsenic oxides, manganese–arsenic oxides, and lead–arsenic oxide (Figure 2).

Further information concerning the effect of arsenic mineralogy on the oral bioavailability of arsenic can be gleaned from the Anaconda monkey study (discussed below). Arsenic mineralogy in fecal material from the monkeys indicated a mineral assemblage similar to that of the preingested soil (*15*). These data suggest that bioavailable arsenic from this soil originates primarily from dissolution of either the surface-bound arsenic fraction or the exterior portion of individual arsenic-bearing grains, rather than through complete dissolution of any discrete arsenic mineral phase. Thus, the data provide mechanistic evidence for the limited bioavailability of arsenic from this soil sample.

Review of in Vivo Database

This section provides a brief review of the in vivo studies that have been conducted to determine the relative bioavailability of lead and arsenic from soil. The test substrates from the soil studies described below constitute the validation samples for ongoing attempts to validate in vitro extraction tests. The following review is not intended to provide a discussion of in vivo study design nor an exhaustive list of all the in vivo studies that have been conducted to date.

In Vivo Database for Lead. Gastrointestinal absorption of lead in humans varies with the age, diet, and nutritional status of the subject as well as with the chemical species and the particle size of lead that is administered. Age is a well-established determinant of lead absorption; adults typically absorb 7–15% of lead ingested from dietary sources, while estimates of lead absorption from dietary sources in infants and children range from 40 to 53% (*16–18*). For the purpose of modeling exposure to lead in soil, the U.S. EPA currently assumes that the absolute bioavailability of lead in diet and water is 50% and that the absolute bioavailability of lead in soil is 30% for children (*19*). This corresponds to a soil RAF of 0.60 (60%) for the bioavailability of soil lead relative to lead in water (i.e., RAF = 0.3/0.5).

In the studies described below, lead absorption from ingested soil was compared to absorption of soluble lead in rats and swine, and the data were used to determine the relative bioavailability of soil lead (results are summarized in Table 1). Soil samples from all of these in vivo studies are available for testing and form the basis for validation of in vitro extraction tests.

EPA Region VIII has developed an oral lead bioavailability assay in a young swine model and has used this model to evaluate relative lead bioavailability in 19 substrates from eight hazardous waste sites. In the young swine model, groups of five swine (5–6 weeks of age) were dosed for 15 days with varying concentrations of lead in soil (<250-μm size fraction) or lead acetate. The swine were dosed twice daily, with the first dose delivered after an overnight fast, and the second dose was delivered in the afternoon after a 4-h fast. The swine were fed 2 h after each dosing. Serial blood samples were collected during the study and analyzed for lead concentration. At the completion of the study, samples of blood, bone (femur), liver, and kidney were collected and analyzed for lead concentration. The resulting data were used to estimate relative lead bioavailability from the test substrates. A more detailed discussion of this model is found in Casteel et al. (*20*).

To date, final study reports have been released for eight sites ((*21–27*, *64*) Table 1). Relative bioavailability estimates have also been developed for lead in unweathered, galena-enriched soil and for paint mixed with soil (*28*, *29*). RAFs for the 19 substrates range from less than 0.01 to 0.90, based on measurement of lead in blood, bone, liver, and kidney (values cited in Table 1 are recommended point estimates based on a combination of these data, with blood data weighted most heavily). In general, the samples that produced the least lead uptake were derived from tailings, smelter slags, and soil from mining sites, while soils from the vicinity of smelters generally yielded higher RAFs. An exception to this observation occurs for carbonate rich soils at mining areas (such as Jasper, MO), where lead carbonates, which yield high lead bioavailability, tend to predominate in the lead mineral assemblage.

Several studies of relative lead bioavailability from soil at mining and smelting sites have been conducted in a weanling rat model (*30–34*). These studies involved dosing groups of five weanling rats for 30 days with varying concentrations of lead-bearing soil (<250-μm size fraction used in all studies, except Dieter et al. who used <38-μm material) or lead acetate in the diet. At the end of the studies, lead concentrations were measured in blood and bone (femur) and various soft tissues (liver, kidney, and brain), depending on the study. RAFs developed from these studies ranged from 0.087 to 0.41 (Table 1), depending on the origin of the various materials studied. As with the swine studies, the lead forms in the samples tested reflect their origin. The sites that produced the lowest lead absorption were mining and tailings sites (primarily lead sulfide and lead sulfate), and those that produced the greatest proportion of bioavailable lead were soils from the vicinity of historical lead and zinc smelters (lead forms present include common soil alteration phases: iron–lead oxides, manganese–lead oxides, lead phosphates, and lead carbonate).

Over the last several years, considerable effort has been applied to developing chemical amendments that would reduce the bioavailability of lead in soils by changing the mineral species of the lead (*5*, *35–38*). These amendments have generally been phosphate based, and they rely on the formation of pyromorphite [$(Pb_5(PO_4)_3X$, X = Cl or OH]-type minerals, which are quite insoluble, to reduce the oral bioavailability of lead. If they are determined to provide long-term reductions in lead bioavailability, these amendments could provide a cost-effective remedial option for lead-contaminated soils. The young swine model discussed above has been used to evaluate the relative bioavailability of lead

TABLE 1: Validation Study Substrates

site/study	test material	lead concn (mg/kg)	arsenic concn (mg/kg)	lead RAF[a,h]	arsenic RAF[a,h]	pH (s.u.)	TOC[i] (%)	ref
	NIST SRM 2711	1162	105	*j*	*j*	7.8	1.25	*55*
	Swine Studies (EPA Region VIII)							
Aspen	soil (berm)	14 200	66.9	0.60	0.62 ± 0.55[b]	*j*	*j*	*24, 45*
Aspen	soil (residential)	3870	16.7	0.61	0.98 ± 0.86[b]	*j*	*j*	*24, 45*
Bingham Creek	tailings (residential soil)	1590	51.2	0.31	*j*	*j*	*j*	*64*
Bingham Creek	tailings (channel)	6330	149	0.28	0.37 ± 0.19[b]	*j*	*j*	*45, 64*
Butte	soil	8585	239	0.19	0.10 ± 0.05[b]	*j*	*j*	*27, 45*
Jasper	soil (HL smelter)	10 800	25.1	0.58	*j*	*j*	*j*	*23*
Jasper	soil (LL yard)	4050	10.7	0.80	*j*	*j*	*j*	*23*
Jasper	soil (HL mill)	6940	16.4	0.79	*j*	*j*	*j*	*23*
Leadville	soil (residential)	7510	203	0.74	−0.08 ± 0.09[b]	*j*	*j*	*25, 45*
Leadville	soil (Fe–Mn lead oxide)	4320	110	0.90	0.28 ± 0.15[b]	*j*	*j*	*25, 45*
Leadville	slag (AV)	10 600	1050	0.18	0.15 ± 0.01[b]	*j*	*j*	*25, 45*
Leadville	tailings (Oregon gulch)	1270	1290	0.06	*j*	*j*	*j*	*25*
Midvale	slag	7895	591	0.17	0.18 ± 0.04[b]	*j*	*j*	*26, 45*
Murray Smelter	slag	11 500	695	0.53	0.51 ± 0.09[b]	*j*	*j*	*21, 45*
Murray Smelter	soil	3200	310	0.71	0.34 ± 0.03[b]	*j*	*j*	*21, 45*
Palmerton	soil (location 2)	3230	110	0.67	0.39 ± 0.09[b]	*j*	*j*	*22, 45*
Palmerton	soil (location 4)	2150	134	0.54	0.52 ± 0.15[b]	*j*	*j*	*22, 45*
NA	NIST paint std + soil	8350	4.8	0.80	*j*	*j*	*j*	*29*
NA	Galena + soil	11 200	4.9	0.01	*j*	*j*	*j*	*28*
Clark Fork	tailings (GK)	152	181	*j*	0.49 ± 0.05[b]	*j*	*j*	*45*
	Swine Studies (Missouri DNR/EPA Region VII)[d]							
Joplin	no. 1—bench control	4300	*j*	0.59	*j*	7.5	4.6	*39*
Joplin	no. 2—bench/1% P, 70 day	4270	*j*	0.38	*j*	8	4.2	*39*
Joplin	no. 6—bench/0.5% P, 1 yr	4610	*j*	0.69	*j*	6.6	3.4	*41*
Joplin	no. 3—field control, 3 mo	5100	*j*	0.67	*j*	3	5.8	*40*
Joplin	no. 4—field/0.5% P, 3 mo	2890	*j*	0.49	*j*	8	6.1	*40*
Joplin	no. 5—field/1% P, 3 mo	4890	*j*	0.45	*j*	5	5.8	*40*
Joplin	no. 7—field/1% P, 1% Fe, 3 mo	4500	*j*	0.82	*j*	3	4.9	*41*
Joplin	no. 8—field control, 18 mo	5200	*j*	0.63	*j*	7.5	5.5	*63*
Joplin	no. 9—field/1% P, 18 mo	4240	*j*	0.39	*j*	6.6	5.5	*63*
	Rat Studies							
Butte	mine waste	3908	*j*	0.093	*j*	3.7	4.1	*48*
Butte	mine waste	3940	*j*	0.225	*j*	3.6	2.61	*48*
Bartlesville	soil	1388	*j*	0.35	*j*	7.0	12.8	*34*
Murray	soil	2090	*j*	0.41	*j*	7.5	4.8	*33*
Copperton	tailings	7220	*j*	0.147	*j*	2.4	0.56	*49*
Copperton	tailings	6890	*j*	0.087	*j*	2.8	1.78	*49*
Bingham Creek	tailings (channel)	10 230	*j*	0.36	*j*	4.9	2.86	*49*
Skagway	ore concentrate	611 000	*j*	0.10[f]	*j*	*j*	*j*	*30*
Skagway	Galena (PbS)	866 000	*j*	0.10[f,g]	*j*	*j*	*j*	*30*
	Swine Studies (U. of Missouri)[e]							
Oklahoma	calcine/soil 1	11 070	11 300	*j*	0.027 ± 0.027[c]	2.6	0.36	*47*
Oklahoma	calcine/soil 2	12 100	17 500	*j*	0.033 ± 0.020[c]	2.6	0.22	*47*
Oklahoma	calcine/soil 3	10 980	13 500	*j*	0.083 ± 0.020[c]	3.1	0.58	*47*
Oklahoma	calcine/soil 4	8430	11 500	*j*	0.221 ± 0.032[c]	3.1	0.41	*47*
Oklahoma	calcine/soil 5	5530	6250	*j*	0.301 ± 0.147[c]	5.7	0.61	*47*
Oklahoma	iron slag 3	3510	1180	*j*	0.287 ± 0.130[c]	7.1	1.58	*47*
Oklahoma	iron slag 4	12 600	5020	*j*	0.30 ± 0.075[c]	7.4	3.38	*47*
Oklahoma	iron slag 5	11 530	4650	*j*	0.164 ± 0.035[c]	7.4	3.22	*47*
	Rabbit Study							
Anaconda	soil	*j*	3900	*j*	0.48	6.6	7.4	*48*
	Monkey Study							
Anaconda	soil (residential)	*j*	410	*j*	0.20	7.8	12	*49*
Anaconda	house dust	*j*	170	*j*	0.28	7.6	42	*49*

[a] All swine-based RBA estimates are recommended point estimates based on blood and tissue data from EPA Region VIII reports. All rat-based RBA estimates are based on measurements of blood lead. [b] Standard deviation from Monte Carlo simulation. [c] Standard deviation across set of swine ($n = 5$) for each substrate. [d] Joplin samples collected and characterized by Missouri DNR. In vivo swine studies conducted at U. of Missouri and funded by EPA Region VII. [e] Oklahoma samples collected and characterized by Nick Basta (Oklahoma State U.). In vivo studies by Stan Casteel (U. of Missouri). [f] Calculated from data in cited reference. [g] No statistical difference was noted between blood and tissue lead based RBA estimates for Skagway ore concentration and PbS. [h] RAF = relative adsorption factor. [i] TOC = total organic carbon. [j] No analysis performed.

from phosphate-amended soils at a site in Joplin, MO. Both the Missouri Department of Natural Resources and EPA Region VII are participants in this project, which is being conducted under the auspices of the U. S. EPA Remedial Technology Development Forum (RTDF). A description of the amendment studies can be obtained from the RTDF Web site at http://www.rtdf.org/iinert.htm.

Control and chemically amended soils evaluated in the swine model to date include (all of the phosphate amendments used phosphoric acid, unless otherwise noted) the following: (1) laboratory control and laboratory-amended soils (1% phosphate in soil at field-capacity moisture content

incubated for 70 days, and 0.5% phosphate incubated at 55 °C for 1 year and subjected to wet/dry cycles every 2–3 weeks); (2) field control and amended soils (0.5 and 1.0% phosphate after 3 months of field weathering, and 1% phosphate as Triple Super Phosphate plus 1% iron as amorphous ferric hydroxide after 3 months of field weathering); and (3) field control and amended soil (1.0% phosphate, 18 months of field weathering).

Results from these animal studies (Table 1 (*39–41, 63*)) suggest that phosphate-based amendments may be effective at reducing lead bioavailability from Joplin soils by 25–40%.

In Vivo Database for Arsenic. Both the cancer slope factor (CSF) and reference dose (RfD) used to assess the cancer risks and other adverse health effects, respectively, that might be associated with oral exposure to arsenic (*42*) were derived from an epidemiological study that characterized health effects in a population of Taiwanese who consumed drinking water containing arsenic (*43, 44*). After ingestion, water-soluble forms of inorganic arsenic are absorbed extensively from the gastrointestinal tract of humans and most laboratory animals. Because most absorbed arsenic is excreted rapidly in urine, urinary arsenic measurements provide a lower limit on the estimate of oral bioavailability. In the studies described below, arsenic absorption from ingested soil was compared to absorption of soluble arsenic in swine, monkeys, and rabbits, and the data were used to calculate the relative bioavailability of soil arsenic.

The bioavailability of soil arsenic has been evaluated in the young swine assay that was designed initially by EPA Region VIII to estimate lead bioavailability. During the lead studies, EPA also evaluated 14 samples for relative arsenic bioavailability. In the young swine model, groups of five swine (5–6 weeks of age) were dosed twice daily for 15 days (as described above) with varying concentrations of arsenic in soil or slag or with sodium arsenate. Urinary arsenic data for the 14 substrates indicate that relative arsenic uptake in these studies varied from near 0 to 50% (Table 1), with the exception of two samples from Aspen, CO that contained arsenic concentrations that were too low (less than 100 mg/kg) to produce reliable RAF values (*45*). Arsenic bioavailability depended on the form of arsenic present in the sample (Table 1 (*45*)). Soil samples containing only smelter waste or tailings in stream sediments contained the greatest amount of bioavailable arsenic (RAFs ranged from 0.34 to 0.52), while samples containing smelter slag produced intermediate values (RAFs ranged from 0.15 to 0.51; Table 1). Soil samples containing only mining waste (Butte), or a mixture of mining waste and smelting waste (two Leadville soil samples), contained the least bioavailable arsenic (RAFs range from −0.08 to 0.28). This finding is not unexpected, because arsenic in mining waste may be present in larger particle sizes and less soluble forms (e.g., sulfides) (*46*).

During the EPA Region VIII swine studies for arsenic bioavailability, arsenic mass recovery was quite low (23 and 36%, respectively, for sodium arsenate and Grant-Kohrs [GK] tailings (*45*)). The reason for this is not clear. Because the basis for this low recovery is unknown, its significance is also unclear. If the mechanism leading to this low recovery is systemic and applies equally to all dosing materials, then the RAFs are expected to be correct. Only if the mechanism affects arsenic recovery differently for different treatment groups would the RAFs be incorrect. This difficulty with the swine model causes considerable uncertainty regarding the accuracy of the RAFs derived from this model. However, at this time, the most comprehensive assessment of relative arsenic bioavailability from a variety of different environmental substrates is based on the young swine model.

The University of Missouri used the same swine protocol to determine relative arsenic bioavailability from eight mineral processing waste samples from Oklahoma. Arsenic mass balance was not evaluated during these studies. The Oklahoma samples are either a calcine material (low pH and TOC) mixed with soils or an iron slag (neutral pH and higher TOC; Table 1). These eight samples produced arsenic RAFs ranging from 0.027 to 0.30 (Table 1 (*47*)).

In one of the first studies of the relative bioavailability of arsenic in weathered soil, New Zealand White rabbits were used to study the oral absorption of arsenic in a soil sample from Anaconda, Montana that had been affected primarily by emissions from a copper smelter (*48*). Mineral speciation results indicated that arsenic in the soil was present primarily as copper–lead–arsenic oxides and iron–arsenic oxides, with minor contributions from enargite (copper–arsenic sulfide), slag, arsenic phosphates, and copper–lead–arsenic silicates. The rabbits were dosed with arsenic in soil as well as receiving soluble sodium arsenate by gavage and by intravenous injection. Based on urinary arsenic data, the RAF was estimated to be 0.48.

Relative arsenic bioavailability from a composite residential soil from the Anaconda smelter site was determined in a second animal model, the monkey (*49*). Although collected from a different area of the site, the mineralogy of the composite soil sample used in the monkey bioavailability study was nearly identical to that of the soil used in the rabbit study. Three female *Cynomolgus* monkeys were administered single oral doses of soil, house dust, or soluble sodium arsenate by gavage or intravenous injection, using a round-robin study design. Based on urinary arsenic data, the RAFs were estimated to be 0.20 for arsenic in soil and 0.28 for house dust. Based on the above studies, it is clear that considerable evidence exists for the reduced bioavailability of arsenic from soil relative to soluble arsenic. However, the available arsenic in vivo database is not as comprehensive or well resolved as that for lead. In addition, the model that has been used most extensively for arsenic, young swine, may have limitations associated with it (e.g., low arsenic mass recovery for unknown reasons), the importance of which remains to be determined.

In Vitro Extraction Tests

Simple extraction tests have been used for several years to assess the degree of metals dissolution in a simulated gastrointestinal-tract environment (*50–52*). The predecessor of these systems was developed originally to assess the bioavailability of iron from food, for studies of nutrition (*53, 54*). In these systems, various metal salts or soils containing metals are incubated in a low-pH solution for a period intended to mimic residence time in the stomach. The pH is then increased to near neutral, and incubation continues for a period intended to mimic residence time in the small intestine. Enzymes and organic acids are added to simulate gastric and small-intestinal fluids. The fraction of lead, arsenic, or other metals that dissolve during the stomach and small-intestinal incubations represents the fraction that is bioaccessible (i.e., is soluble and available for absorption). For example, the European Standard for Safety of Toys (*55*) provides for an extraction test to evaluate the bioaccessibility of eight metals (including arsenic and lead) from children's toys. The European method involves extraction of the particular metal (toy material reduced to $<500\ \mu m$ in size, at a liquid-to-solid ratio of 50:1) in pH 1.5 (HCl) fluid at 37 ± 2 °C for 2 h. This method has been in use since 1994 by the 18 member countries of the Comite European de Normalization (CEN) to regulate the safety of toys.

Variation in the bioaccessibility of arsenic, chromium, nickel, cadmium, and lead, as a function of liquid-to-solid ratio, was evaluated by Hamel et al. (*56*). These authors determined that bioaccessibility in synthetic gastric juice was affected only slightly by changes in the liquid-to-solid ratios in the range of 100:1 to 5000:1 (mL/g). Ruby et al. (*52*)

demonstrated that for a set of seven soils that had been evaluated for relative lead bioavailability in a weanling rat model, the stomach phase of the in vitro test at a pH value of either 1.3 or 2.5 correlated with RAFs from the in vivo model ($r^2 = 0.93$ at both pH values, $p < 0.01$). More recently, a revised version of the extraction test (different test cell and stirring method) developed in the laboratory of Dr. John Drexler, at the University of Colorado at Boulder, has indicated that data from the stomach phase of the test correlates well with in vivo data for samples used in a series of young swine studies conducted by EPA Region VIII and the University of Missouri ($r^2 = 0.85$, $n = 15$ (*57*)). These results indicate that the extent of lead dissolution in the acidic stomach environment of the extraction test is predictive of relative lead bioavailability in two animal models (weanling rats and young swine). Finally, concentrations of lead, extracted from house dust by the method of Ruby et al. at pH 3.0 (stomach phase only), correlated with blood lead values for children living in the homes from which the particular house dust was collected ($r^2 = 0.79$, $n = 7$ (*58*)).

Further research by Dr. Drexler has resulted in a streamlined extraction test for estimating relative lead bioavailability: 1-h extraction (mixing by end-over-end rotation at 37 °C) of 1 g of soil (<250-μm size fraction) in 100 mL of buffered (HCl and glycine) pH 1.5 solution. Preliminary results for this test appear to correlate well with relative lead bioavailability values from the EPA Region VIII swine studies (*59*). A formal validation of this extraction test in three independent laboratories is currently being conducted (described below).

For arsenic, the correlation between in vitro and in vivo estimates of relative arsenic bioavailability is less clear, primarily due to a less comprehensive and reliable in vivo database (discussed above). Recent research in the laboratory of Dr. Nick Basta (Oklahoma State University) indicates that results from both stomach-phase (pH 1.8, 60 min in a stirred flask at 37 °C) and small-intestinal-phase (pH 5.5, bile acids, pancreatic enzymes, 60 min in a stirred flask at 37 °C) extractions correlated equally well with RAFs from the EPA Region VIII young swine model for 13 mining-related samples ($r^2 = 0.69$ and 0.67, respectively, $p < 0.01$ (*47*)). As with lead, these data suggest that the extent of arsenic dissolution during an acidic gastric-like extraction is predictive of RAFs in the young swine model.

The Solubility/Bioavailability Research Consortium (SBRC)

To further the development and acceptance of in vitro methods for estimating metals bioavailability from soil, a stakeholder group was formed in January 1997, titled the Solubility/Bioavailability Research Consortium (SBRC). The SBRC is a collaborative effort among scientists from academia, government agencies, consulting firms, and industry. It was convened to develop and validate in vitro methods for estimating the relative bioavailability of metals in contaminated soils. Funding for the SBRC has been provided primarily by the industrial members of the group. The New Jersey Department of Environmental Protection (DEP) and the Massachusetts DEP have participated by providing input to this research effort.

The initial goal of the SBRC is to develop and validate an extraction test that correlates with in vivo measurements of relative lead bioavailability from soil in an immature swine model (discussed above). Once this has been accomplished, the extraction test will be available for use in deriving site-specific human exposure estimates. Data have been collected simultaneously for arsenic and used to evaluate the applicability of the test for arsenic bioavailability determination. Every effort is being made to develop tests that are simple, convenient, and reproducible and to disseminate these test protocols for general use in risk assessment.

The SBRC's ongoing research includes an in vitro method validation study for lead and arsenic in three independent laboratories (each extracting blind triplicates of each in vivo tested material), ongoing laboratory research to elucidate the factors that control arsenic bioaccessibility under simulated gastric and small-intestinal conditions, and efforts to develop a more comprehensive in vivo database for relative arsenic bioavailability from soil. Future work will most likely involve application of the existing in vitro test, or development of alternative tests, to other inorganic elements of human health concern (e.g., beryllium, cadmium, chromium, mercury, and nickel).

Discussion

One of the thorny issues facing the SBRC is the question of what constitutes "validation" of a bioaccessibility test for developing site-specific exposure values for use in human health risk assessment. The polar viewpoints appear to advocate either that such a test must be "validated" extensively against appropriate animal models for each new element or that—given our current understanding of the mechanisms that control metals bioavailability from soil—the existing bioaccessibility tests are more appropriate than animal studies and do not require "validation" at all. Between these viewpoints are more moderate perspectives, which might include "validation" against animal models for specific elements, or classes of elements (e.g., cations versus anions when dissolved in solution), rather then rigorous "validation" studies for every element. Validation efforts for lead and arsenic have depended on large in vivo databases developed over the past decade. Similar databases are not available for other metals, requiring that alternative approaches be considered.

All of the animal models currently in use for bioavailability assessment, with the possible exception of monkeys, have substantial anatomical and physiological differences from humans (*60, 61*), and none of these models has been validated against estimates of metal absorption in either children or adults (only one soil sample has ever been studied for oral metals bioavailability in humans [lead only (*62*)], although rough oral absorption estimates can be derived from site-specific exposure studies that have been conducted in human populations). Thus, it is possible that the animal models used most extensively to date (rats and swine) may produce relative bioavailability estimates that are not equal to those in humans.

The research to date indicates that the extent of lead and arsenic dissolution in the acidic environment of the stomach is predictive of relative oral bioavailability of these elements in animal models. Although lead and arsenic are absorbed through the intestinal epithelium, these results suggest that the rate-controlling step in oral lead and arsenic absorption is dissolution in the stomach rather than absorption across the intestinal epithelium. As a result, the extraction test required to simulate the rate-controlling process is quite simple. Given this simplicity, the test is likely to be more reproducible (i.e., have smaller discrepancy between replicate measurements) and sensitive (i.e., capable of being used at lower soil lead and arsenic concentrations) than a well-designed in vivo study for the same soil. Based on the apparent feasibility of developing extraction tests for bioavailability assessment, the observed range of relative lead and arsenic bioavailability, and the great difference in cost between in vivo and in vitro models (a factor of 500–1000), the development of such tests appears to be a worthwhile goal.

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